AGAR-AGAR.

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The preparation of agar by the older methods is well known to be a tedious operation, which consumes much valuable time. The product obtained is seldom, if ever, quite transparent; while not infrequently troublesome precipitates which not only mar the appearance of the medium but render it unsuitable for the finer classes of work, develop after sterilization.

The use of powdered agar, which has been in the market for two or three years, because of its ready solubility, simplifies the process and greatly shortens the time required in the preparation of the medium; but for some reason, doubtless because of the scant notice which has been given to the matter in the literature, it does not yet seem to have come into general use. To call attention to the powdered form, and to report a method for obviating the appearance of secondary precipitates in the tubes, on sterilization, was the object of a paper by the writer published in the first number of the Journal of Applied Microscopy.

The method then described materially lessened the time and labor required in the preparation of agar and gave a perfectly transparent product. Subsequent efforts, aided by a suggestion obtained from an article by Dr. Ravenel, in the June number of the Journal, have enabled us to shorten the time limits from two and one-half hours to one hour, counting from the time of the receipt of the meat in the laboratory until the last drop of the completed medium has passed through the filter, and yet obtain average results; while by deferring filtration until after the first sterilization a perfectly transparent medium is obtained. In the latter event from half to three-quarters of an hour suffices for the initial preparation, exclusive of the time required for

sterilization in bulk, but a half hour more is required on the following day for re-heating and filtering. The process is as follows:

Rub up 10 grams each of powdered agar and Witte's powdered peptone, and 5 grams of sodium chloride, in a porcelain-lined saucepan, with just sufficient water to thoroughly moisten the powder and form a thin paste; add gradually, while stirring the mixture, 500 cc. of water; place on a gas stove, interposing a piece of asbestos board or wire gauze between the saucepan and flame, and heat the mixture until the agar is dissolved, stirring occasionally to prevent burning on the bottom of the dish. If the paste made with cold water is properly rubbed up, so as to break down all the lumps and moisten all the agar, solution will be practically complete by the time the boiling point has been reached, so that two or three minutes brisk boiling suffices.

With the aid of a meat press extract the juice from 500 grams (one pound) of lean meat, and add the juice to 500 cc. of water. Mix this "flesh-water" with the agar solution—which now should have cooled sufficiently not to coagulate the albumin in the flesh-water, but still be hot enough to remain fluid—and carefully neutralize with a 4 per cent solution of caustic soda.

After neutralization boil the mixture until all the coagulable albumin in the flesh-water has been coagulated and comes to the surface, leaving a clear fluid beneath. Again test the reaction, and, if need be, correct it; add sufficient boiling water to supply any loss that may have occurred through evaporation, and filter through paper. To insure rapid and complete filtration without the necessity of reheating the mass I distribute the solution in three or four filters, using coarse, folded paper, pass sufficient boiling water through each filter to wash away loose lint and thoroughly heat the funnels just previous to commencing the filtration of the agar. With good paper and proper attention to detail filtration is usually accomplished in from ten to fifteen minutes.

While filtration is in progress sterilize or boil a tube of the filtrate. If it remains clear after heating, and when cold is free

from sediment and only slightly opalescent, the entire filtrate may be immediately run off into tubes and sterilized. But if a precipitate should make its appearance either on heating or while cooling, the filtrate should be sterilized in mass and allowed to stand in the sterilizer with the light turned low or out until the precipitate collects together at or near the bottom of the flasks when the agar may be reheated and refiltered; this time, with the confident expectation that the filtrate will be and will subsequently remain transparent. Or, if preferred, the agar may be run off into cylindrical deposit glasses, sterilized therein, and allowed to stand in the sterilizer, as before, until the sediment has settled to the bottom after which the clear fluid may be syphoned off, or allowed to cool and cut off with a knife and the portion containing the sediment be discarded, or filtered, according to amount.

Usually, on account of the liability to secondary precipitates, and because the agar is never so transparent when filtered immediately as it is when the filtration is deferred until after the first sterilization, I do not filter at once, but merely strain out the coarser flocculi by running the medium through losely packed cotton, sterilize in flasks, allow the flasks to stand in the sterilizer and slowly cool, and wait until the following day before filtering through paper. Filtration is then still more rapid, if care is taken to bring the temperature of the mass up to the boiling point in the sterilizer before commencing the filtration, and the product is always transparent.

The coarser precipitates which occur on sterilization are usually due to the coagulation of albumin which has escaped coagulation at the time of the preparation of the medium; but the troublesome ones are of more doubtful origin; probably they consist, in the first place, of very fine flocculi which pass through the filter on the first filtration, and, in the second place, of salts which are held in solution during the first filtration but which as a result of changes in the reaction, oxidation, or because of lessened solubility in the cold medium and their presence to supersaturation, are deposited as the medium cools.

But whatever their nature and cause I have been unable to avoid their appearance altogether save by the method just detailed. When present in only small amount and sterilization is not too much prolonged, (ten minutes) if the tubes are *quickly* cooled they cause no perceptible sediment and only a slight opalescence in the finished product and are then really not objectionable, though I always prefer to have my media perfectly transparent, if possible.

Eggs are not needed to clear the agar when made by the above process, the albumin in the meat juice being sufficient for the purpose.

If it be desirable to make agar from bouillon it is only necessary to rub up the powdered agar with a little of the cold bouillon to a paste and then gradually add the balance of 500 cc. thereof, and boil until solution—which quickly takes place—is complete; add the balance (500 cc.) of the bouillon; stir in the whites of two eggs and boil until the egg albumin is coagulated and rises to the surface leaving the clear solution beneath, and then filter, as before. As, however, the agar can be made from the flesh-water almost as readily and quickly as the bouillon itself, there is little inducement for the use of previously prepared bouillon.

Meat extract can also be substituted for the flesh-water. Formerly I used from 20 to 30 cc. of Valentine's meat juice per liter, but more recently I use but 10 to 15 cc. which quantity I find sufficient. I prefer Valentine's to other extracts that I have tried as it makes a lighter colored agar and seems to be free from resistant spores, as no more care is required in the sterilization of the media made from it than from meat itself. If 10 cc. of meat extract (or meat juice as Valentine terms it), be added to 500 cc. of water and substituted for the flesh-water the process is the same as with the latter, save that egg albumin must be added to clear the medium if it be desired to filter before sterilization. Meat extract, being readily kept on hand, is more convenient than meat for the preparation of media, but some organisms do not seem to thrive so well upon the media thus made.

The precaution of first moistening the agar and peptone with a small quantity of cold water or cold bouillon, as the case may be, and rubbing to a smooth paste free from lumps, must not be omitted. If stirred directly into a hot solution—and to a less extent if stirred directly into a large quantity of cold water, without previous moistening—the agar rolls up into little lumps and is almost as difficult of solution as the finely cut pieces of shred agar.

If a meat press is not at hand the flesh-water can be made in the ordinary way either by macerating finely minced meat in cold water for a few hours, or by digesting for a shorter time at a higher temperature.